

Characterization and comparison of general esterases from two field populations of the grasshopper *Oxya chinensis* (Thunberg) (Orthoptera: Acridoidea)

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Abstract: Malathion susceptibility in the two populations of the grasshopper *Oxya chinensis*, collected from Linyi of Shanxi Province and Xuzhou of Jiangsu Province, China, was determined. General esterases from the two populations were characterized and compared. LD₅₀ of the Xuzhou population (13.00 µg/g body weight) was 2.80-fold higher than that of the Linyi population (4.64 µg/g body weight). Inhibition studies of general esterases using four inhibitors, including paraoxon, malaoxon, eserine, and carbaryl, indicated that most general esterases in the two populations were B-type. Kinetic studies showed that the Michaelis-Menten constant (K_m) and the maximal velocity (V_{max}) of general esterases from the Xuzhou population were higher than that from the Linyi population, using α -naphthyl acetate (α -NA), α -naphthyl butyrate (α -NB), β -naphthyl acetate (β -NA) as substrates. The esterase activities in females of the Xuzhou population were 2.02, 1.58, and 1.28-fold higher than those of the Linyi population, using α -NA, α -NB and β -NA as substrates, respectively, and in males they were 2.71, 1.67, and 1.33-fold higher in the Xuzhou population than in the Linyi population. The spectrum of esterase activities showed that *O. chinensis* individuals with high esterase activities were more in the Xuzhou population than those in the Linyi population using the three selected substrates. We speculated that esterases in the Xuzhou population may be biochemically different from those in the Linyi population, and it might be attributed to the different geographic distributions, ecological environment and nutrition resources in the two localities. In addition, the biochemical differences might also be due to the difference in insecticides selective pressure on the two populations of *O. chinensis*.

Key words: *Oxya chinensis*; general esterases; malathion susceptibility; enzyme kinetics; esterase inhibition

1 INTRODUCTION

The grasshopper *Oxya chinensis* (Thunberg) (Orthoptera: Acridoidea) is distributed over a broad range of latitude from Far East coastal region of Russia, Korea, China to Japan and Vietnam. The insect is a prominent agricultural pest in China and represents a most widespread pest, which is commonly and abundantly found in rice paddies, sugar cane, maize, gramineous plants and other crop fields (Zheng, 1993) and brings about great losses for agricultural production.

Due to different ecological environment and geographic distribution, *Oxya chinensis* in different regions have some remarkable variations in growth, development and propagation (Ji, 1999), which may accompany by different genetic structures. Furthermore, a moderate geographical barrier might significantly restrict the gene exchange among populations, which may result in the accumulation of

local genetic diversity within a population and the development of genetic differentiation among various populations (Li *et al.*, 2004).

Synthetic insecticides, especially organophosphate (OP) insecticides, are often used in management programs to control *O. chinensis* in China owing to their properties of ready degradation and low residues (Shen *et al.*, 1988; Sun and Peng, 1991). Recently, it has been noticed by pesticide applicators that control of *O. chinensis* populations in some localities in China has become increasingly difficult with the organophosphates. Numerous studies have demonstrated that esterases play important roles in conferring or contributing to insecticides resistance in insect and other arthropod species (Qiao *et al.*, 2003; Li *et al.*, 2003). Esterases cause insecticide resistance primarily via sequestration of insecticides by large amounts of esterases present in resistant insects (Devonshire and Moores, 1982; Hemingway and Karunatne, 1998).

Understanding population's genetic background can shed light on effective control of *O. chinensis*. The

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objectives of this study were to (1) compare malathion susceptibility in two field populations of *O. chinensis*; (2) classify general esterases of *O. chinensis*; (3) compare biochemical properties of general esterases in the two populations.

2 MATERIALS AND METHODS

2.1 Insects

The fifth-instar nymphs of *O. chinensis* were collected from Xuzhou of Jiangsu, an coastal province in East China, and Linyi of Shanxi, an inland province in North China, in 2003. The habitat of the Xuzhou population (XZ) consists of a reservoir-shore field, whereas the habitat of the Linyi population (LY) consists of flood river desert sand. All collected specimens were stored at -20°C for short time stock.

2.2 Chemicals

Bicinchoninic acid solution (BCA), eserine (hemisulfate salt), fast blue B salt (O-dianisidine, tetrazotized), α -naphthol, β -naphthol, α -naphthyl acetate (α -NA), β -naphthyl acetate (β -NA), α -naphthyl butyrate (α -NB) were purchased from Sigma Chemical Co. (St. Louis, MO). Malathion (99.5% pure), paraoxon (90% pure), malaoxon and carbaryl (99% pure) were purchased from Chem Service (West Chester, PA). Bovine serum albumin (BSA) was purchased from Bio-Rad Laboratories (Hercules, CA). Triton X-100 was purchased from Sangon.

2.3 Insecticide bioassay

The susceptibility of *O. chinensis* to malathion was evaluated using a microsyringe injection method. Six different concentrations of malathion were prepared in acetone as a solvent. A sample of 16–24 fifth-instar nymphs of *O. chinensis*, which was designated as a replicate, was individually injected with 4 μL of malathion solution or acetone (control) in the abdomen between the second and third sterna. Each bioassay was carried out with six malathion doses and a solvent control; each dose or control was repeated three times. Mortality was determined after the treated nymphs were maintained at room temperature for 24 h.

2.4 Assay of general esterase activity

Thorax of a fifth-instar nymph of *O. chinensis* was homogenized in 0.9 mL ice-cold 0.1 mol/L phosphate buffer (pH 7.5) containing 0.3% (V/V) of Triton X-100. The homogenates were centrifuged at $15\,000 \times g$ for 20 min at 4°C , and the supernatants were transferred to fresh tubes and used as enzyme sources. General esterase activities were assayed by the method of van Asperen (1962) with some modifications by Zhu and He (2000), using α -NA, α -NB and β -NA as substrates. Briefly, 15 μL of appropriately diluted enzyme preparation was incubated in a final reaction volume of 150 μL in 0.1 mol/L phosphate buffer (pH

7.5) containing 0.27 mmol/L substrate at 37°C for 30 min. Reactions were stopped by adding 50 μL of fast blue B-SDS solution. After 15 min, absorbance was determined using a V_{\max} kinetic microplate reader and SOFTmax computer software (Molecular Devices, Menlo Park, CA).

2.5 Kinetic analysis of general esterases

Kinetic parameters of general esterases were determined using three selected substrates, *i. e.* α -NA, α -NB and β -NA, as previously described (Zhu and He, 2000). 15 μL of appropriately diluted enzyme preparation, as previously described in the assay of general esterase activity, was used in each assay. Final concentrations for all three substrates were 6.25, 12.5, 25, 50, 100, 200 and 400 mmol/L. The Michaelis constant (K_m) and the maximal velocity (V_{\max}) were estimated by Hanes transformations (Bell and Bell, 1988).

2.6 In vitro inhibition of general esterases

Inhibition of general esterases by paraoxon, malaoxon, carbaryl and eserine was studied in the female and male from the two populations. The inhibition reaction was started by incubating 10 μL of the enzyme preparation with 10 μL of each inhibitor at approximately 24°C for 5 min. The remaining esterase activity was determined immediately using α -NA as a substrate as previously described (Zhu and He, 2000).

2.7 Protein assay

Protein contents of enzyme preparations were determined according to Smith *et al.* (1985), using BSA as a standard. Measurements were performed with the microplate reader at 560 nm (Zhu and Clark, 1994).

3 RESULTS

3.1 Comparison of malathion susceptibility

Comparison of malathion susceptibility of *O. chinensis* in two populations was presented in Table 1. Although the ratio calculated with dividing the LD_{50} of the Xuzhou population by that of the Linyi population was only 2.8, there was significantly difference in LD_{50} between the the Xuzhou and Linyi population. The 95% confidence limits (CL) of LD_{50} were not overlapping between the two populations. The Xuzhou population was 2.8-fold less susceptible to malathion than the Linyi population.

3.2 Assay of general esterases

There were significant differences in esterase specific activities between the Xuzhou and Linyi population (Table 2). General esterase specific activities in females of the Xuzhou population were 2.02, 1.58 and 1.28-fold, and in males were 2.71, 1.67 and 1.33-fold higher than those in the females and males of the Linyi population, when α -NA, α -NB or β -NA was used as a substrate, respectively.

Table 1 Comparison of malathion susceptibility of the fifth-instar nymphs of *Oxya chinensis* collected from Linyi and Xuzhou populations

Population	<i>N</i> *	Slope ± <i>SE</i>	χ ²	<i>P</i> **	LD ₅₀ (μg/g body weight) (95% CL)	LD ₅₀ ratio
Xuzhou	390	1.57 ± 0.07	4.14	0.99	13.00(10.11 – 16.41)	2.8
Linyi	487	2.43 ± 0.06	8.09	0.95	4.64(4.05 – 5.41)	–

* Number of the *O. chinensis* nymphs tested in each bioassay. ** *P* ≥ 0.05 indicates a significant fit between the observed and expected regression lines in a probit analysis.

Table 2 Comparisons of general esterase activities [μmol/(min·mg)] using α-NA, α-NB and β-NA as substrates in Linyi and Xuzhou populations of *Oxya chinensis*

Sex	α-NA		α-NB		β-NA	
	LY	XZ	LY	XZ	LY	XZ
♀	0.151 ± 0.036 a	0.304 ± 0.074 a *	0.174 ± 0.055 a	0.277 ± 0.060 a *	0.204 ± 0.044 a	0.262 ± 0.063 a *
♂	0.119 ± 0.031 b	0.321 ± 0.065 a *	0.170 ± 0.055 a	0.284 ± 0.062 a *	0.191 ± 0.041 a	0.254 ± 0.051 a *

Results are presented as the mean ± *SD* (*n* = 32). Means within columns followed by the same letter are not significantly different (*P* > 0.05) using Student's *t*-test. * Means within rows are significantly different (*P* < 0.05) using Student's *t*-test.

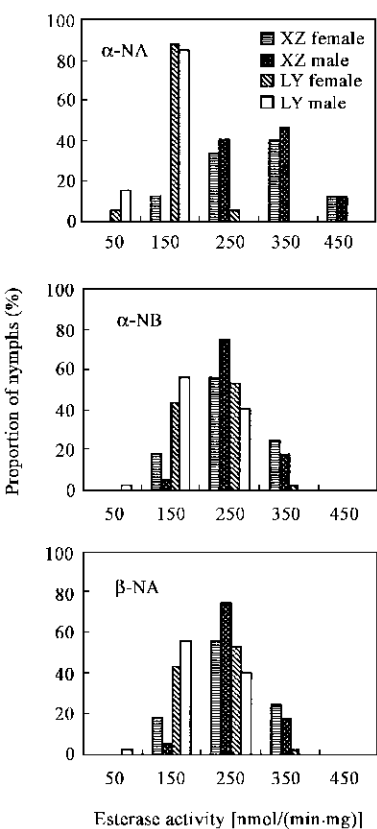


Fig. 1 Frequency distributions of *Oxya chinensis* with esterase activity using α-NA, α-NB, and β-NA as a substrate respectively in Xuzhou (XZ) and Linyi (LY) populations

The esterase activity was individually determined in 32 nymphs of *O. chinensis* for each sex of each population using the esterase microassay.

3.3 Esterase activity spectrum

Fig. 1 showed the different esterase activities spectrum between the two populations using α-NA, α-NB and β-NA as substrates. There were more individuals containing high esterase specific activity in

the Xuzhou population than those in the Linyi population for both females and males using the three selected substrates.

3.4 General esterase kinetics

Fig. 2 showed the effects of the concentrations of the three substrates, α-NA, α-NB and β-NA on the activities and Hanes plots (inserted) for kinetics of esterases of Xuzhou and Linyi populations. Enzyme kinetics studies indicated that esterases from the two populations have significant differences in *K_m* value and *V_{max}* value (Table 3 and 4). *K_m* value of general esterases hydrolyzing α-NA, α-NB, β-NA in the females of the Xuzhou population were 1.2, 1.5, 1.3-fold, and in males were 1.1, 1.4, 0.8-fold, respectively, higher than those in the Linyi population. *V_{max}* values of general esterases in the females of the Xuzhou population were 2.0, 1.6, 1.6-fold, and in male were 1.9, 1.7, 1.2-fold, respectively, higher than those in the females and males of the Linyi population. Among three substrates tested, α-NA appeared to be the most favorable substrate for general esterases of *O. chinensis*, having the lowest *K_m* values in both populations. In contrast, α-NB is not a preferred substrate for esterases, having the highest *K_m* values in both populations.

3.5 In vitro inhibition of general esterases

Two organophosphates (paraoxon and malaoxon) and two carbamates (eserine and carbaryl) were used for *in vitro* inhibition of general esterases (Fig. 3). Paraoxon was the most potent inhibitor of the esterases. Paraoxon at 10⁻⁵ mol/L inhibited 91.1% and 93.9% of the esterase activities in females and 93.1% and 92.6% in males within the Xuzhou and Linyi population, respectively. For malaoxon, only 64.6% and 50.5% of general esterase activities in females and 53.5% and 57.8% in males within the Xuzhou and Linyi population, respectively, were inhibited at the same concentration. Carbaryl at the same concentration

inhibited 33.7% and 28.8% of general esterase activities in females and 34.7% and 28.6% in males for the Xuzhou and Linyi populations. Eserine was the least potent inhibitor of the esterases. Eserine at 10^{-5} mol/L inhibited nothing of general esterase activities in the Linyi population and in females for the Xuzhou population and only 3.8% of general esterase were

inhibited in males for the Xuzhou population (Fig.3). There were significant differences in the pI_{50} (the negative logarithm of the medium inhibition concentration) values for malaoxon, carbaryl and eserine in females, paraoxon, carbaryl and eserine in males between the two populations (Table 5) by Student's t -test.

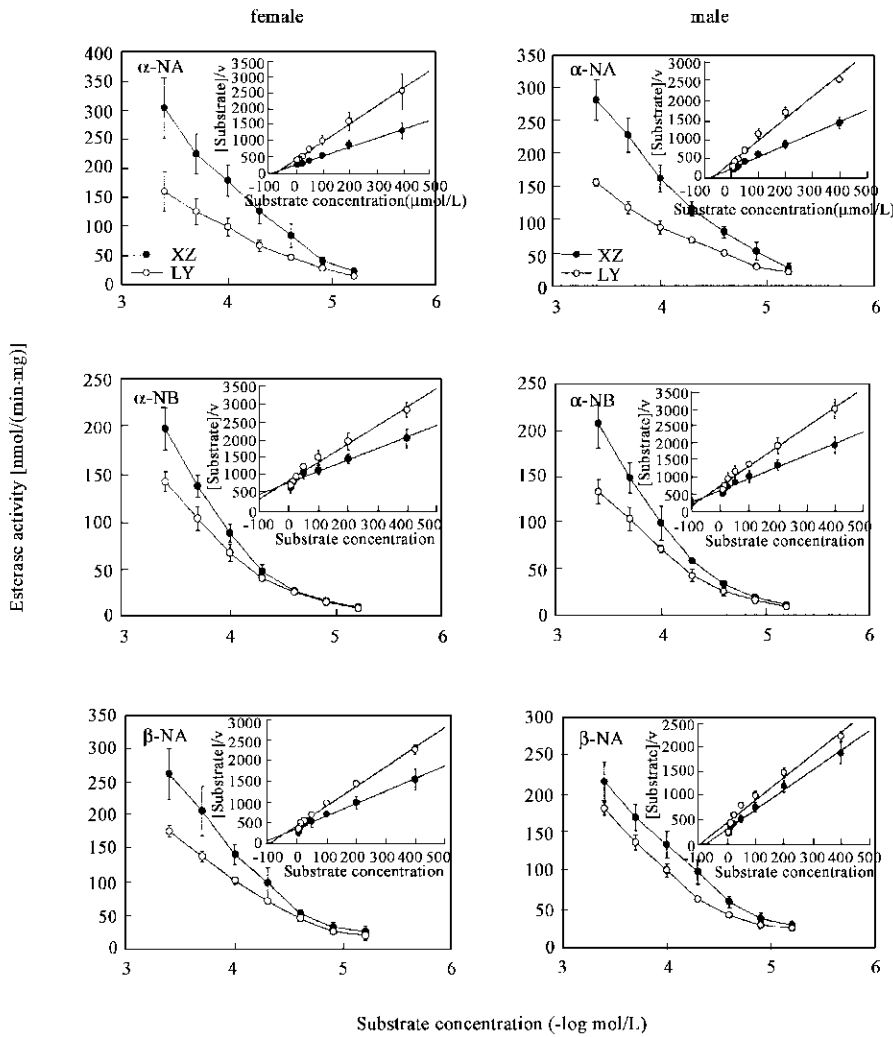


Fig. 2 Effect of substrate concentration on the hydrolysis of α -NA, α -NB, and β -NA by esterases from Xuzhou (XZ) and Linyi (LY) populations
Each point represents the mean of four determinations ($n=4$). Vertical bars indicate SD of the mean. The secondary plots (inserted) are Hanes plots of $[S]$ vs $[S]/v$ for esterase hydrolyzing α -NA, α -NB or β -NA.

Sex	α -NA		α -NB		β -NA	
	LY	XZ	LY	XZ	LY	XZ
♀	81.7 ± 17.3 a	101.8 ± 27.1 a	167.1 ± 8.1 a	249.6 ± 33.1 a*	93.1 ± 9.8 a	120.0 ± 15.3 a*
♂	73.5 ± 6.4 a	84.0 ± 5.6 a*	131.8 ± 2.6 b	182.5 ± 14.2 b*	95.4 ± 4.9 a	75.6 ± 7.2 b*

Results are presented as the mean ± SD ($n=4$). Means within columns followed by the same letter are not significantly different ($P>0.05$) using Student's t -test. * Means within rows are significantly different ($P<0.05$) using Student's t -test. The same for Table 4 and 5.

Table 4 V_{max} values [$\mu\text{mol}/(\text{min}\cdot\text{mg})$] using α -NA, α -NB, β -NA as substrates in the Linyi and Xuzhou populations of *Oxya chinensis*

Sex	α -NA		α -NB		β -NA	
	LY	XZ	LY	XZ	LY	XZ
♀	0.19 ± 0.05 a	0.37 ± 0.08 a*	0.20 ± 0.02 a	0.32 ± 0.04 a*	0.21 ± 0.01 a	0.33 ± 0.04 a*
♂	0.18 ± 0.01 a	0.33 ± 0.03 a*	0.18 ± 0.02 a	0.30 ± 0.05 a*	0.22 ± 0.01 a	0.25 ± 0.03 b

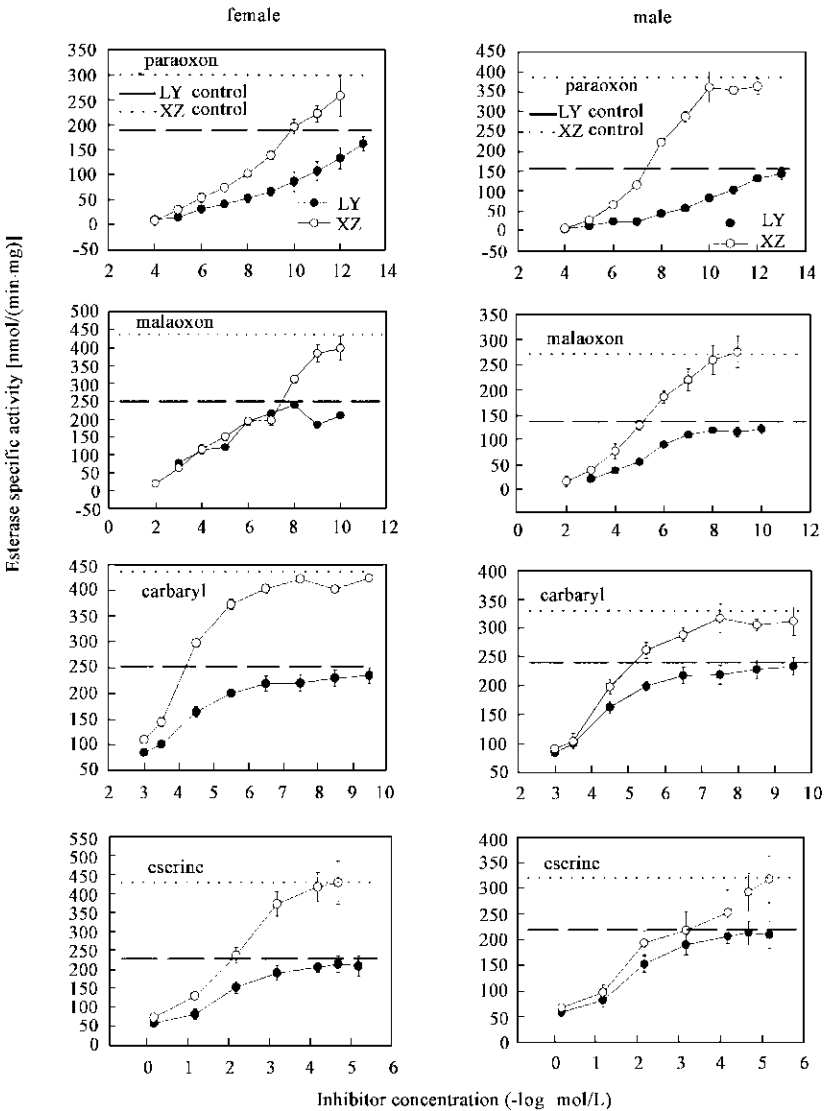


Fig. 3 Inhibition of general esterases from the Linyi (LY) and Xuzhou (XZ) populations of *O. chinensis* by four selective inhibitors, paraoxon, malaoxon, carbaryl and eserine at room temperature (approx. 24°C)
Vertical bars indicated *SD* of the mean of four determinations ($n = 4$).

4 DISCUSSION

General esterases are commonly classified into three types based on their interactions with organophosphates (Aldridge, 1953). A-type esterases are not inhibited by organophosphates but degrade organophosphates as their substrates, whereas B-type esterases are readily inhibited

by organophosphates. In contrast, C-type esterases do not interact with organophosphates. Based on this classification, most of general esterases from both the Xuzhou and Linyi populations of *O. chinensis* were B-type because they were very sensitive to inhibition by organophosphate compounds, especially paraoxon. Based on that 1×10^{-5} mol/L paraoxon almost completely inhibited the esterase activities (Fig. 3), but did not

cause inhibition to arylesterases (A-type) (de Malkenson *et al.*, 1984), we estimated that about 91.1% and 93.9% of general esterases in the females and 93.1% and 92.6% in the males for the Xuzhou and Linyi populations, respectively, were B-type esterases. Because B-type esterases can be further classified into carboxylesterases and cholinesterases based on their different responses to inhibition by eserine, our studies also suggested that carboxylesterases were predominant in the composition of general esterases in the two populations of *O. chinensis*. It had been reported that 1×10^{-7} mol/L

eserine blocked cholinesterase activity completely in *Myzus persicae* (Sudderuddin, 1973) and *Musca domestica* (van Asperen, 1962), and it also partially inhibited carboxylesterase activity at higher concentrations (Sudderuddin, 1973). Based upon the criteria that cholinesterases can be completely inhibited by 10^{-7} mol/L eserine, it is suggested that almost all of B-type esterases were carboxylesterases for the Xuzhou and Linyi populations (Fig. 3).

Table 5 pl_{50} values for paraoxon, malaoxon, carbaryl and eserine in *in vitro* inhibition to esterases of Linyi (LY) and Xuzhou (XZ) populations of *Oxya chinensis*

Inhibitor	Population	pl_{50}		Regression coefficient, r		r significance, P	
		Female	Male	Female	Male	Female	Male
Paraoxon	LY	10.34 ± 0.98 a	10.12 ± 0.90 a	< - 0.97	< - 0.99	< 0.0001	< 0.0001
	XZ	9.39 ± 0.38 a	7.75 ± 0.17 b*	< - 0.98	< - 0.99	< 0.0001	< 0.0001
Malaoxon	LY	4.47 ± 0.05 b	4.94 ± 0.32 a	< - 0.98	< - 0.97	< 0.001	< 0.0001
	XZ	6.20 ± 0.30 a	5.20 ± 0.36 a*	< - 0.97	< - 0.98	< 0.0001	< 0.0001
Carbaryl	LY	3.73 ± 0.08 b	3.72 ± 0.07 b	< - 0.99	< - 0.98	< 0.001	< 0.001
	XZ	3.97 ± 0.09 a	4.02 ± 0.20 a	< - 0.98	< - 0.98	< 0.001	< 0.001
Eserine	LY	1.49 ± 0.11 b	1.49 ± 0.12 b	< - 0.98	< - 0.98	< 0.01	< 0.01
	XZ	1.75 ± 0.09 a	2.03 ± 0.18 a	< - 0.98	< - 0.96	< 0.01	< 0.01

Xuzhou and Linyi populations belong to the different distribution areas of *O. chinensis*. The Xuzhou population is distributed in the reservoir-shore area which consist of a maize field and a weed field, where *O. chinensis* is feeding mainly on maize and gramineous plants and insecticides, mainly organophosphates, were applied in recent years for protecting crop. However, the Linyi population belongs to a river flood area, which is a desolate sand, some weed including primarily gramineous plants are abundant food supply for *O. chinensis*, and insecticides were seldom used for *O. chinensis* control. Furthermore, the higher temperature and humidity in Xuzhou are more favorable to the survival of *O. chinensis* than in Linyi. The distance between the two localities is about 1 200 km. In addition, *O. chinensis* has low migratory capabilities and they often fly in a small scale, which make genetic differentiation among various populations easier to occur. However, in morphology there are no significant differences between the two populations.

Our bioassay results revealed only 2.8-fold decreased susceptibility to malathion in the Xuzhou population as compared with the Linyi population. The marginally decreased malathion susceptibility in the Xuzhou population is likely due to the different ecological breeding habitat, climate and nutrition resources. The significantly different K_m values on esterase activity from the two populations suggested that their catalytic abilities toward the same substrate were different and that the

different esterase activities in *O. chinensis* based on comparisons of general esterases activity and spectrum in two populations may be caused by the differentiation of the two populations. Higher V_{max} values in the Xuzhou population also showed its esterase activity was different from the Linyi population. Consequently, we speculated that esterases in the Xuzhou population may be biochemically different from those in the Linyi population, and it might be attributed to the different geographic distributions, ecological environment and nutrition resources in the two localities. In addition, that the biochemical differences might also be due to the different selective pressures of insecticides on Xuzhou and Linyi populations. Therefore, improving ecological environment plays an important role in effective control of *O. chinensis*.

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中华稻蝗两地理种群酯酶特性的比较研究

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摘要: 对采自江苏徐州和山西临猗两个种群中华稻蝗进行了马拉硫磷敏感性的生物测定, 同时对两个种群的酯酶特性进行了比较研究。生物测定结果表明, 徐州种群的 LD_{50} 值($13.00 \mu\text{g/g}$ 虫重)是临猗种群($4.64 \mu\text{g/g}$ 虫重)的 2.8 倍; 用对氧磷、马拉氧磷、西维因及毒扁豆碱等四种抑制剂对该两个种群的酯酶的体外抑制研究表明, 两个种群所含酯酶大都为 B 型酯酶; 酯酶动力学研究结果表明, 徐州种群动力学参数米氏常数 (K_m 值)和最大反应速度 (V_{\max} 值)均较临猗种群为高; 用 α -乙酸萘酯(α -NA)、 α -丁酸萘酯(α -NB)和 β -乙酸萘酯(β -NA)三种底物测定酯酶活性, 在雌性稻蝗中, 徐州种群比临猗种群分别高 2.02、1.58 和 1.28 倍, 雄性中则分别高 2.71、1.67 和 1.33 倍; 对两个种群酯酶活性频率分布进行比较, 徐州种群中酯酶活性高的个体数远大于临猗种群。我们推测徐州种群酯酶的生化特性可能不同于临猗种群, 这可能与地理分布、生态环境和食物条件不同有关, 杀虫剂选择压力不同可能也起一定的作用。

关键词: 中华稻蝗; 酯酶; 马拉硫磷敏感性; 酶动力学; 酯酶抑制

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